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EXTRINSIC AND INTRINSIC NEURAL CONTROL OF PYLORIC SPHINCTER PRESSURE IN THE DOG

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SUMMARY

1. In chloralose–urethane-anaesthetized dogs a manometric assembly was inserted via a gastrostomy to monitor pyloric pressure with a sleeve sensor. Antral and duodenal contractions were monitored with both manometric side holes and serosal strain gauges.

2. Subserosal silver wire electrodes were placed in the antrum 5 cm orad and the duodenum 3 cm aborad to the pylorus to facilitate field stimulation of intramural nerves.

3. The pylorus exerted spontaneous tone (10·8 ± 4·8 mmHg) with phasic contractions occurring at a rate varying from 1–5 min^{-1} and, at times, with a superimposed higher frequency up to 15 min^{-1}. Atropine (30 $\mu g\ kg^{-1}$ I.V. and 10 μg I.A.) reduced and tetrodotoxin (50–100 μg I.A.) enhanced the phasic activity significantly.

4. Bilateral cervical vagal section had no consistent influence on pyloric motility.

5. Stimulation of the distal ends of the cervical vagal nerves at low frequencies (0·2–0·5 Hz, 1–3 ms, 20 V) induced phasic pyloric contractions, which were abolished by atropine or hexamethonium (10 mg kg^{-1} I.V. and 1 mg I.A.). Higher frequencies (> 0·7 Hz) of stimulation inhibited both phasic and tonic contractions and this inhibition was unaffected by atropine, hexamethonium, phentolamine (1·5 mg kg^{-1} I.V. and 100 μg I.A.) or propranolol (1 mg kg^{-1} I.V. and 100 μg I.A.). All neural responses were blocked by tetrodotoxin (50–100 μg I.A.).

6. Duodenal field stimulation (0·2–5 Hz, 0·5 ms, 40 V) induced strong phasic and tonic contractions in the pylorus. This excitation was blocked by atropine, hexamethonium, tetrodotoxin (50–100 μg I.A.) or duodenal transection orad to the stimulating electrodes.

7. Antral field stimulation (0·5–1 Hz, 0·5 ms, 40 V) completely abolished phasic activity in the pylorus and reduced tonic activity, regardless of whether the contractile activity was spontaneous or induced by neural stimulation. This inhibitory action was unaffected by atropine, hexamethonium or propranolol but was blocked by tetrodotoxin and antral transection aborad to the stimulating electrodes. Phentolamine attenuated the inhibitory effect of antral field stimulation on pyloric motility.

8. It is concluded that the distal canine pylorus exhibits myogenic tone and phasic activity which is modulated by extrinsic and intrinsic nerve pathways. Vagal nerves contain fibres, activated by different stimulus parameters which can either excite or inhibit pyloric activity. Activation of antral nerves inhibits pyloric activity, with both non-adrenergic, non-cholinergic and phentolamine-sensitive pathways contributing to this inhibitory response. Activation of duodenal intrinsic nerves activates a chain of orally projecting cholinergic nerves which enhances pyloric activity. The pyloric muscle should be considered as a sphincter because of its myogenic activity and special neural control.

INTRODUCTION

Schulze-Delrieu & Shirazi (1983) recently reviewed the anatomy of the pyloric region, pointing out that, in the dog as well as in human and cat, the pyloric sphincter region contains a special inner muscle consisting of two muscle rings forming an intermediate and a distal sphincter. The two rings join as a torus at the lesser curvature.

Recording the activity of this pyloric structure separately from the activity of the overlying antrum is difficult, which may explain why, since the investigations of Langley (1898), there has been much controversy as to the function of the pylorus and the regulation of pyloric activity. Whereas some authors have considered the pylorus only as an extension of the antrum, others have considered it a sphincter (Brink, Schlegel & Code, 1965). Studies in man, dog, cat and rat have demonstrated a pyloric high-pressure zone with a variable width of 0·5–1·5 cm (Brink *et al.* 1965; Behar, Biancani & Zabinski, 1979; Heddle, Downton, Dent, Toouli & Maddern, 1986). This zone has been reported to exhibit characteristic responses to hormonal and neural stimulation, distinguishing it from the antral region (Fisher & Cohen, 1973; Anuras, Cooke & Christensen, 1974; Behar *et al.* 1979).

The neural mechanisms mediating control of pyloric motility are even less well understood. Some studies suggest that intrinsic as well as extrinsic nerves play a significant role in pyloric activity (Behar *et al.* 1979; Mir, Telford, Mason & Ormsbee, 1979; Lerman, Jacobowitz, Mason, Garber & Ormsbee, 1981; Reynolds, Ouyang & Cohen, 1984). Since the recording technique used in these studies did not discriminate between activities arising from the inner muscle ring and the overlying antrum, these findings refer to the entire thickness of the muscle wall in the pyloric region.

In the present study we have used an adaptation of the sleeve manometric technique (Dent, 1976) to monitor intraluminal pyloric pressure, concurrently with side-hole manometric and strain gauge recording of duodenal and antral motor activity. Monitoring of the latter two activities enables, in certain circumstances, local pyloric activity to be distinguished from that of the overlying muscle.

The aims of this study were to investigate further the myogenic activity of the pylorus and the neural pathways which modulate pyloric motility and to attempt (1) to determine if this pyloric muscle initiates myogenic tone and phasic activity, (2) to investigate the influence of different cervical vagal nerve stimulation parameters on pyloric motility and (3) to evaluate the influence of antral and duodenal intramural nerve stimulation on the pylorus, and their possible participation in the regulation of pyloric contractions.

Preliminary results of this work have been presented at meetings of the Gastroenterology Society of Australia and the American Gastroenterology Association and published as an abstract (Allescher, Dent, Daniel, Kostolanska & Fox, 1987).

METHODS

Animal preparation

Thirty-nine mongrel dogs (mean weight ± S.D. 18·0 ± 4·1 kg) of either sex were anaesthetized with pentothal (50 mg I.V.) and then given α-chloralose (60 mg kg^{-1} I.V.) and urethane (500 mg kg^{-1} I.V.). Level III anaesthesia (assessed by the absence of the eye-lash and corneal reflex, no change in heart rate or blood pressure in response to pain stimulus) was maintained with bolus injections of sodium pentobarbitone (30 mg, I.V.) as required. The animals were ventilated through a tracheal tube using a Harvard dog respirator 607 (Harvard Apparatus, Edenbridge, U.K.). The right femoral artery and vein were cannulated to enable recording of systemic blood pressure and the systemic administration of drugs. The cervical vagi were carefully dissected free and cut during ($n = 26$) or before ($n = 2$) the experiment. In eleven additional experiments both vagal nerves stayed intact. After vagal transection, insulated bipolar stimulating electrodes were applied to the distal ends of both vagal trunks and stimulated simultaneously. Neither duodenal nor antral field stimulation was influenced by vagal section. All results given for duodenal and antral field stimulation are obtained after vagal section.

A mid-line incision was made in the abdomen and the gastroepiploic artery cannulated, at a point 1 cm from the pylorus in both directions, allowing close intra-arterial injection either to the pylorus and proximal duodenum or to the gastric antrum. The perfusion area was determined before the main experiment with a flush of Krebs solution (1–2 ml) and after the experiment by injecting black ink into the intra-arterial cannulae. Four strain gauges were sutured to the serosa 2 and 5 cm proximal and distal to the pylorus and orientated to record circular smooth muscle activity. Silver wire electrodes (1·0 cm long, 1 cm apart) were inserted subserosally 5 cm orad to the pylorus around the proximal gastric strain gauge and between the two duodenal strain gauges 3–4 cm aborad to the pylorus. Square-wave electrical pulses were delivered by a Grass S 88 and a S 09 for field stimulation and vagal stimulation respectively.

Pressure measurement

Intraluminal pressures were recorded using a manometric assembly incorporating a sleeve sensor 3 cm long and 3·5 mm in diameter. The assembly was inserted through a small fundic gastrostomy and passed through the pylorus and brought out via a small duodenostomy approximately 15–20 cm aborad to the pylorus (Fig. 1). The sleeve was positioned such that recordings from side holes above and below the sleeve registered only antral or duodenal pressure respectively. To facilitate this positioning, antral or duodenal motility was elicited by field stimulation via the silver electrodes.

The manometric lines were perfused with degassed distilled water, using a low-compliance pneumohydraulic perfusion system (Arndorfer pneumo-hydraulic capillary perfusion system, Greenfield, WI, U.S.A.), at a reservoir pressure of 380 mmHg, giving a constant flow rate of 0·3 ml min^{-1}. Pressures were recorded with either Cobe (Cobe, Lakewood, CO, U.S.A.) or Bell and Howell type 4-327-7 (Bell and Howell Limited, Pasadena, CA, U.S.A.) pressure transducers.

All data (force, pressure) was recorded on a Beckman R 411 dynograph. The sensitivity of the recorder was set such that 200 mmHg (26·67 kPa) represented full pen excursion and the pressure transducer baselines were checked regularly throughout the experiment to ensure accurate measurement of basal pyloric tone. Amplification of the strain-gauge signals was calibrated such that maximal field stimulation or 75% of the maximal response to acetylcholine injection represented full pen excursion.

Drugs

Drugs were injected I.A. via gastroepiploic cannulae in 0·1–1·0 ml volumes and flushed with 1·0 ml heparinized Krebs solution. (Composition (in mM): NaCl, 137·4; $MgSO_4$, 1·2; KH_2PO_4, 1·2; glucose, 11·1; $NaHCO_3$, 21·9; $CaCl_2$, 2·5; KCl, 4·5.) Drugs used in this study were acetylcholine,

atropine, hexamethonium, propranolol and tetrodotoxin (TTX) (Sigma, St Louis, MO, U.S.A.), phentolamine mesylate was a gift of Pfizer (Brooklyn, NY, U.S.A.).

The concentration of blockers used in this study were atropine sulphate (30 μg kg^{-1} I.V. plus 10 μg I.A.), hexamethonium (10 mg kg^{-1} I.V. and 1 mg I.A.), phentolamine (1·5 mg kg^{-1} I.V. and 100 μg I.A.) and propranolol (1 mg kg^{-1} I.V. and 100 μg I.A.).

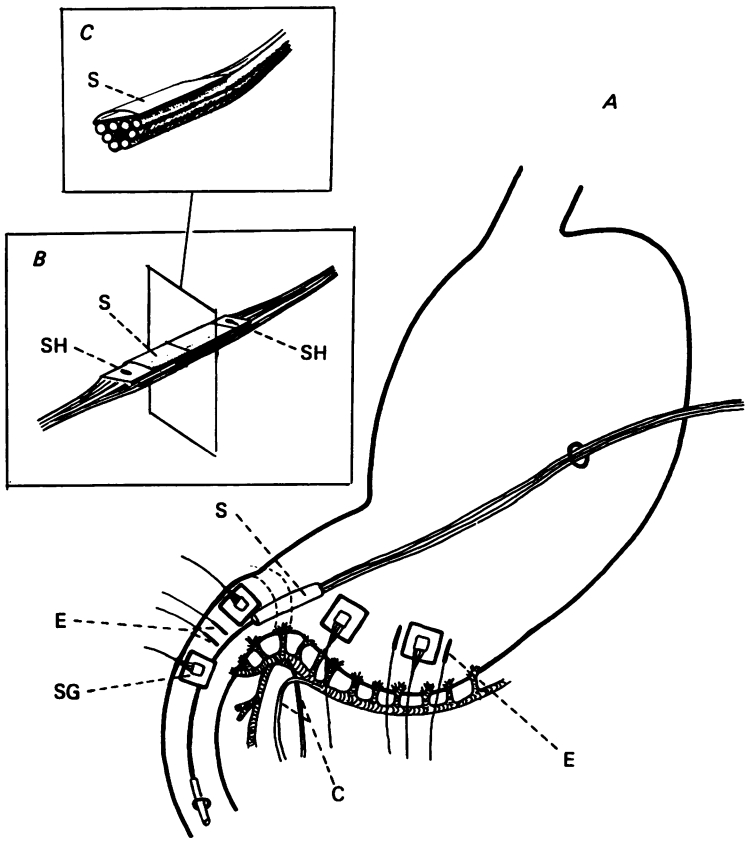


Fig. 1. Schematic drawing of the experimental model and the recording device used. The tubing, consisting of a multiluminal side-hole catheter and a sleeve (S) recording device, was inserted through a gastrostomy. Strain gauges (SG) and silver wire electrodes (E) were placed in the antrum and the duodenum. Intra-arterial cannulae (C) for close intra-arterial injections to the pylorus and the antrum are shown, while the enlargement shows the sleeve recording device (S) and the antral and duodenal side holes (SH).

Analysis of records

Pyloric pressure was referenced to antral pressure recorded from the antral side hole at the same time. The frequency of basal phasic pyloric contractions and those during stimulation was determined by counting each pressure wave of more than 10 mmHg occurring in a period of 2 min and calculating the mean frequency per minute. A motility index (the product of the number of phasic pyloric contractions per minute and their mean amplitude in millimetres of mercury) was then derived. A computerized micro-planimeter system (Laboratory Computer Systems, Inc., Cambridge, MA, U.S.A.) was used to determine the area between the pyloric pressure tracing and the reference baseline. This area, expressed as pressure in millimetres of mercury per minute,

represents the mean pyloric pressure during this time. In addition, a measure of pyloric tone was obtained by tracing the area between the baseline and the bottom points of the phasic pyloric pressure waves. The difference between the whole area and the tonic area gives a theoretical value for the mean phasic pyloric pressure per minute. This approach to data evaluation is illustrated in Fig. 2.

Statistical analysis

All data are given as the mean ± S.D.; n represents the number of independent observations in different animals. Statistical significance was tested using one-way analysis of variance and significance accepted when $P < 0.05$.

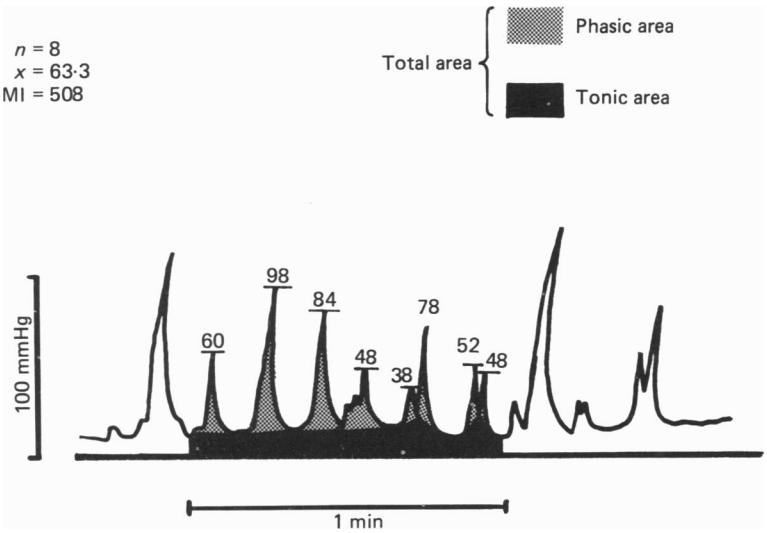
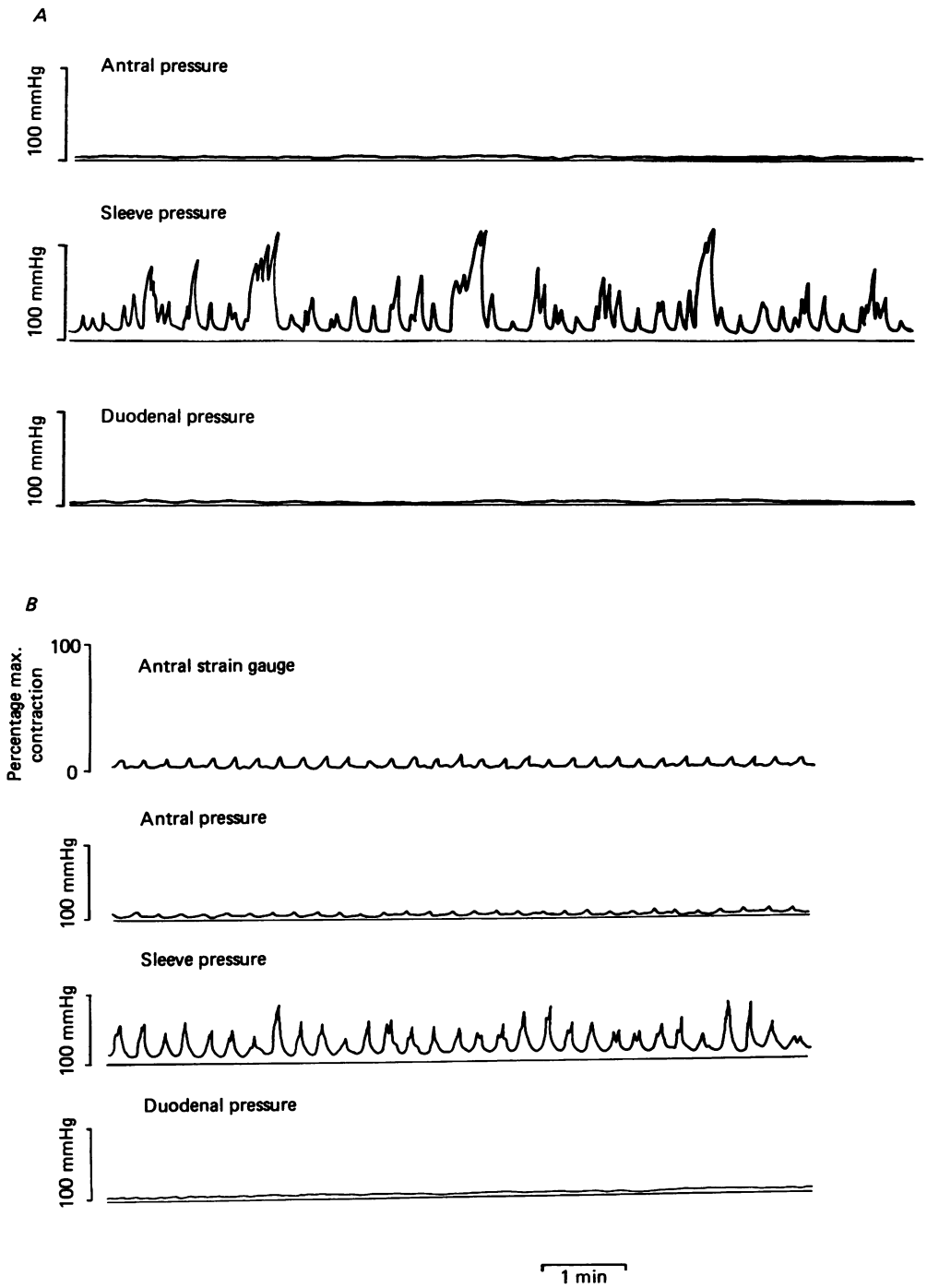


Fig. 2. Schematic example of the data evaluation of the pyloric pressure tracings. All pressure waves of more than 10 mmHg are counted and the amplitudes over the baseline determined. The mean number of the pressure waves per minute gives the mean phasic frequency. The product of the total number and the mean of the amplitude (mmHg) gives the motility index (MI). The whole area under the waves consists of a filled area (tonic area) and a stippled area (phasic area).

RESULTS

Basal activity of the pylorus

The basal activity of the pylorus resulted in a resting intraluminal pressure of 10·8 ± 4·8 mmHg ($n = 28$) and spontaneous pressure waves. These pyloric pressure waves occurred, almost exclusively, without any pressure changes in the terminal antrum or in the proximal duodenum and can therefore be classified as isolated pyloric pressure waves (IPPWs). Furthermore, since the pyloric pressure waves occurred without any indication of antral or duodenal activity recorded by the strain gauges above and below the sleeve at the normal gain setting, they could be classified as isolated pyloric contractions (IPC) (Fig. 3*A*). However, when the amplification of the strain gauge signal was increased 10- to 100-fold, small contractions at a rate of 4–5 min^{-1} in the antrum and approximately 17 min^{-1} in the duodenum were recorded by the strain gauges. Hence, the definition of isolated pyloric contractions (IPCs) is


A
Antral pressure
100 mmHg
Sleeve pressure
100 mmHg
Duodenal pressure
100 mmHg
B
Percentage max. contraction
100
0
Antral strain gauge
Antral pressure
100 mmHg
Sleeve pressure
100 mmHg
Duodenal pressure
100 mmHg
1 min

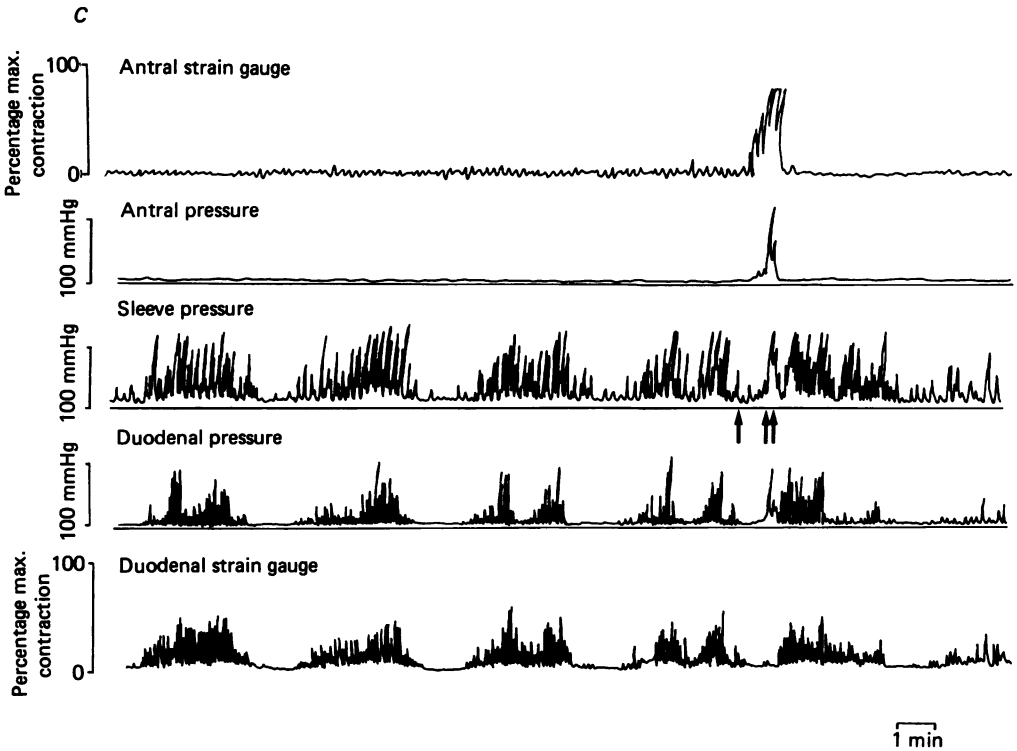


Fig. 3. Dynograph recording showing different examples of pyloric basal activity. *A* shows isolated pyloric contractions without any contractile activity in the antrum and the duodenum. *B* shows a typical pattern of close link between antral motor activity and pyloric motor activity. *C* shows a typical recording of a close link between duodenal and pyloric motor activity interrupted by antral activity inhibiting duodenum and pylorus (single arrow) followed by a contractile wave reaching the pyloric region (double arrow). (Pressure in antral side hole – Antral pressure; pressure in duodenal side hole – duodenal pressure.)

relative and depends on the amplification used in the measurement of antral and duodenal motility. Nevertheless, pyloric contractions due to contractions of adjacent or overlying antral or duodenal muscle could be easily distinguished, on the basis of their relative magnitudes, from those which were not.

Occasionally, there was a close relationship between pyloric activity and antral (Fig. 3*B*) or duodenal activities (Fig. 3*C*). The mean number of the pyloric pressure waves (IPPWs) was $5{\cdot}2 \pm 1{\cdot}6$ min^{-1} and their mean amplitude $30{\cdot}7 \pm 10{\cdot}2$ mmHg (mean $\pm$ S.D.) ($n = 28$). The IPPWs from different segments of the record showed a distribution between the normal antral (3–5 min^{-1}) and the normal duodenal (17–18 min^{-1}) frequency. The pyloric contractions typically showed a biphasic pattern or consisted of only monophasic contractions, often at a higher frequency than these in a biphasic pattern (Fig. 3*A* and *C*). Atropine reduced the phasic activity of the pyloric region significantly ($n = 16$, $P < 0{\cdot}05$) (Fig. 4).

The effect of intra-arterial tetrodotoxin (TTX) on pyloric motor activity

The intra-arterial administration of 50–100 μg TTX to the pylorus caused an increase in basal pyloric motor activity (Fig. 4). This effect was even more pronounced after prior administration of atropine. The contraction pattern became more regular after TTX with the average number of pyloric contractions being $5{\cdot}5 \pm 1{\cdot}3$ min^{-1}. The average amplitude increased from $30{\cdot}7 \pm 10{\cdot}2$ mmHg ($n = 28$) in the basal state, to $54{\cdot}2 \pm 19{\cdot}0$ mmHg after TTX ($n = 10$; $P < 0{\cdot}01$). TTX administration blocked all pyloric motor responses to vagal stimulation and both antral and duodenal field stimulation (see below).

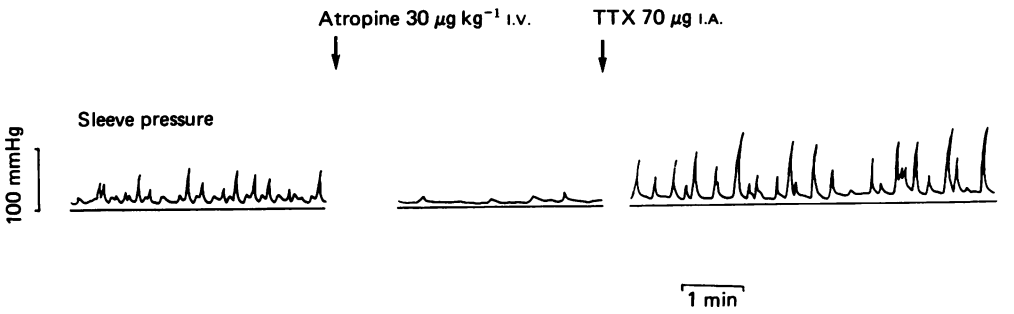


Fig. 4. Dynograph recording showing the pyloric activity in the basal state, after administration of atropine (30 μg kg^{-1} I.V. and 10 μg I.A.) and after intra-arterial injection of 70 μg tetrodotoxin, respectively. After atropine the phasic activity is significantly reduced, without any change in the basal tone. After TTX the pylorus shows isolated contractions with a frequency of 5·2 contractions min^{-1} and significantly increased amplitude. Note the superimposed frequent contractions on the basic contractile pattern.

The influence of vagal transection and stimulation on pyloric motility

Vagal transection was associated with a variable pattern of change in basal pyloric motility. Most commonly ($n = 12$) transection caused an increase in basal phasic activity. However, when basal activity was high, vagal transection had no effect ($n = 10$) or caused a decrease in phasic activity of the pylorus ($n = 4$).

The effect of stimulating the distal vagal stumps was investigated over a wide range of different stimulus frequencies (0·1–10 Hz) and durations (0·2–3 ms). Variation of the stimulus frequency altered the response from excitation at low (0·1–0·5 Hz) (Fig. 5*A*) to inhibition at higher frequencies (1–5 Hz) (Fig. 5*B* and *C*). Stimulus–response analysis at the low frequencies showed that the pyloric region responded with one contraction to each stimulus pulse (Fig. 5*A*). Even though stimulation using these low parameters caused excitation of the pylorus, it suppressed the normal phasic motility pattern. Atropine sulphate ($n = 6$) or hexamethonium ($n = 6$) blocked the excitatory effect of the stimulation. However, the inhibition of pyloric motor activity by high-frequency vagal stimulation was not affected by atropine ($n = 8$), hexamethonium ($n = 6$) or both ($n = 4$), nor by propranolol

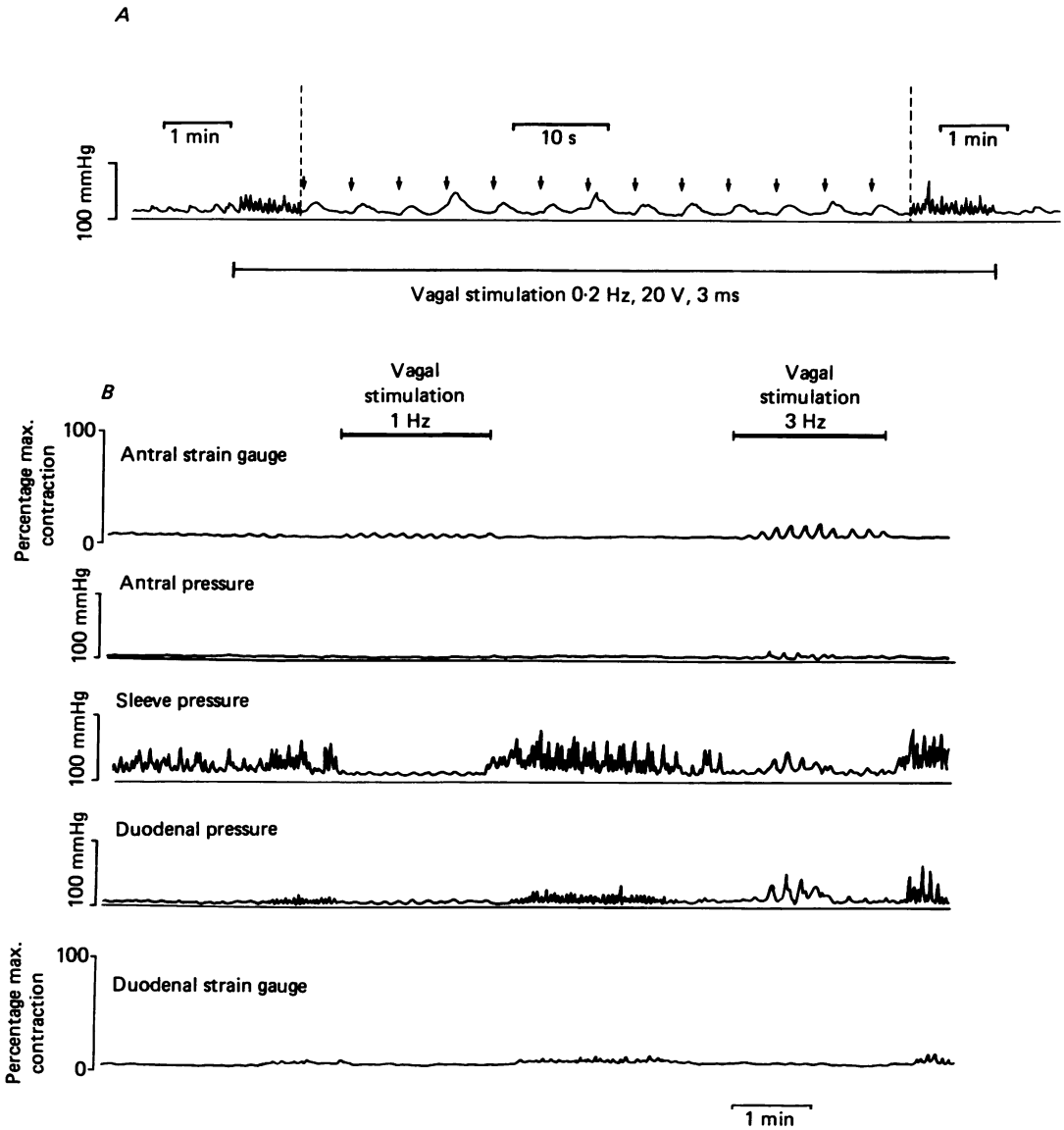


Fig. 5. For legend see p. 26.

($n = 5$) or phentolamine ($n = 5$), but was abolished by I.A. administration of 50–100 μg TTX to the pylorus ($n = 5$).

When the pylorus was activated by duodenal field stimulation (see below) this activity could also be suppressed by vagal stimulation using the inhibitory stimulus parameters ($n = 16$) (Fig. 5*C*).

Vagal stimulation at higher stimulus frequencies (> 3 Hz) and long duration (> 1 ms) caused an initial inhibition of pyloric motor activity followed, after a few seconds, by antral contractions which produced pressure waves detected by the

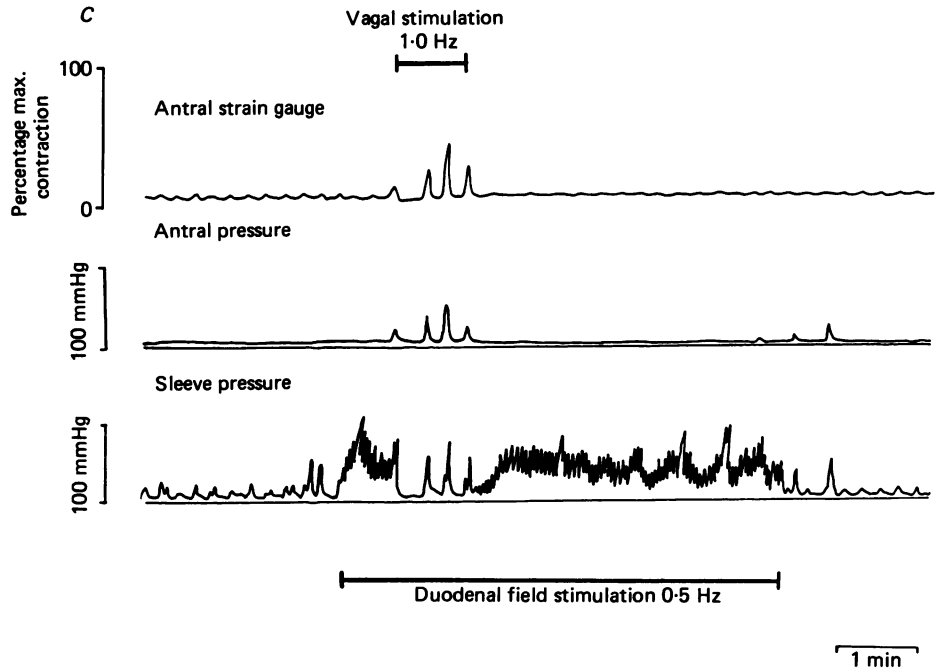


Fig. 5. *A*, excitatory responses of the pylorus to low-frequency vagal stimulation (0·2 Hz, 3 ms, 20 V) at normal paper speed and with extended time scale. The stimulation pulses are indicated by the arrows. No motor responses were recorded in antrum or duodenum (not shown). *B*, inhibitory effects, of vagal stimulation at higher frequencies (1–3 Hz, 3 ms, 20 V), on basal activity and on pyloric motor activity stimulated by duodenal field stimulation (*C*).

antral side hole, the sleeve and the strain gauges. A clear distinction as to whether the pyloric activity remained inhibited or was subsequently incorporated in the antral motor activity was consequently not possible (Fig. 5*C*).

The influence of duodenal field stimulation on pyloric activity

Field stimulation of the duodenum caused an almost immediate pyloric activation (Fig. 6) ($n = 16$). The pyloric motor response consisted of a significant increase in pyloric tonic pressure, mean phasic activity, the average number of the pyloric pressure waves and the motility index (Table 1 and Fig. 7). The excitation from duodenal field stimulation was most effective at frequencies from 0·5 to 1 Hz and showed a phase locking between the frequencies of pulses applied at low frequencies ($< 0{\cdot}7$ Hz) and contractions. The mean frequency, therefore, of the phasic pyloric contractions was maximal at 0·5 Hz and decreased at higher frequencies. It was possible to 'drive' the pylorus with different frequencies, according to the stimulus parameters applied, in the range from 0·1 up to 0·6 Hz and up to 1 Hz in some experiments (Fig. 7).

Atropine ($n = 16$) or hexamethonium ($n = 9$) abolished the activation of the pyloric region by duodenal field stimulation while propranolol ($n = 7$) or phen-

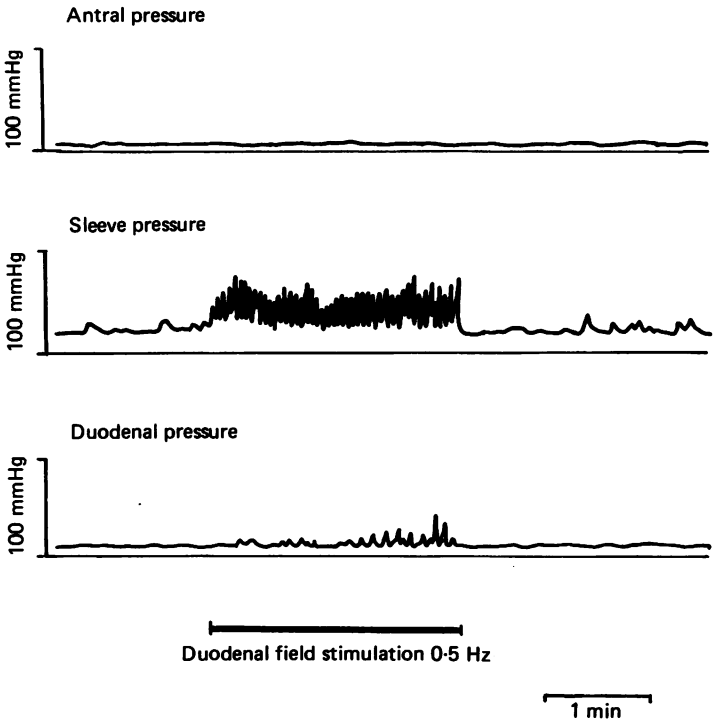


Fig. 6. Dynograph recording of the pyloric motor responses due to duodenal field stimulation (0·5 Hz, 0·5 ms, 40 V). The mean contraction frequency is 30 min^{-1} representing a contraction to every stimulus pulse. Motor activity recorded at the duodenal strain gauge (not shown) resembled that in duodenal pressure tracing.

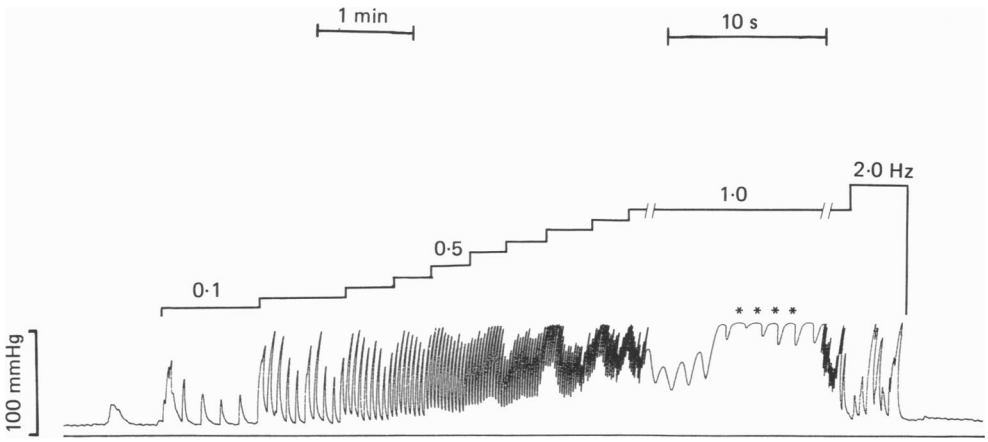


Fig. 7. The effects of duodenal field stimulation with increasing frequency from 0·1 to 2·0 Hz. The pyloric contractions are clearly related to pulse frequency up to 1·0 Hz. At 1 Hz the response is partly off scale (*).

TABLE 1. Pyloric motor response due to duodenal field stimulation

	Control	0·2 Hz	0·5 Hz	1·0 Hz	3·0 Hz	5·0 Hz
n ...	28	7	24	11	9	7
Total area (mmHg min^{-1})	16·2 ± 6·9	30·1 ± 10·3**	51·1 ± 18·4***	50·4 ± 17·2***	35·2 ± 14·5***	28·2 ± 14·5*
Tonic component (mmHg min^{-1})	10·8 ± 4·8	17·5 ± 5·5*	32·7 ± 11·8***	31·0 ± 12·6***	21·5 ± 9·6**	18·1 ± 7·9*
Phasic component (mmHg min^{-1})	5·6 ± 3·2	12·8 ± 6·2**	18·0 ± 12·0***	19·4 ± 10·9***	13·7 ± 7·5**	10·1 ± 7·7
Motility index	159·7 ± 72·1	493·7 ± 215·1***	1199·9 ± 611·9***	976·0 ± 740·6***	638·7 ± 393·7***	422·2 ± 361·9*
Number of contractions per min	5·2 ± 1·6	10·6 ± 1·9***	19·1 ± 6·0***	13·8 ± 8·3***	11·2 ± 4·0***	7·9 ± 3·8*

Values obtained as described in Methods. The values give mean ± S.D. of *n* independent observations. All values given were obtained after vagal transection. Statistical significance was tested by one-way analysis of variance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

TABLE 2. Effect of antral field stimulation on stimulated pyloric motor activity

	Control before	Duodenal FS	Duodenal FS + antral FS	Duodenal FS	Control after
Total area (mmHg min^{-1})	16·1 ± 7·4	57·3 ± 21·4***	15·1 ± 8·6	51·4 ± 20·4***	15·7 ± 6·4
Tonic component (mmHg min^{-1})	10·6 ± 5·5	32·7 ± 12·1***	9·9 ± 5·7	30·6 ± 12·8***	9·8 ± 4·7
Phasic component (mmHg min^{-1})	5·8 ± 3·1	24·6 ± 16·8***	5·2 ± 4·5	19·8 ± 12·5***	5·9 ± 2·7

Values were obtained as described in Methods. The values give mean ± S.D. of fourteen independent observations. All values were obtained after vagal transection. Statistical significance was tested by one-way analysis of variance (***$P < 0.001$) against control. FS, field stimulation.

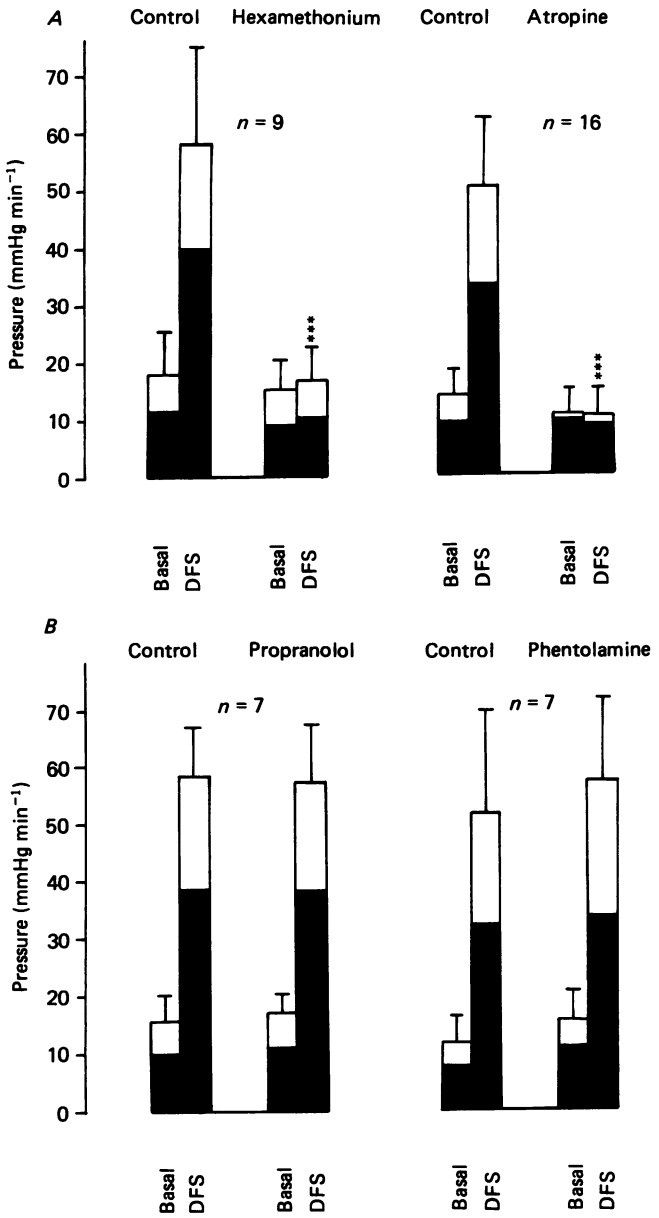


Fig. 8. Histograms showing total pressure, tonic (filled) and phasic component (open) of the pyloric motor activity (mean ± S.D.) in the basal state and during duodenal field stimulation (DFS) at 0·5 Hz before and after application of *A*, atropine (30 μg kg^{-1} I.V. and 10 μg I.A.) or hexamethonium (10 mg kg^{-1} I.V. and 1 mg I.A.); *B*, phentolamine (1·5 mg kg^{-1} I.V. and 100 μg I.A.) or propranolol (1 mg kg^{-1} I.V. and 100 μg I.A.). After atropine or hexamethonium the response to duodenal field stimulation is blocked (***$P < 0{\cdot}001$). Note the reduced phasic activity after atropine with no effect on the basal tone.

tolamine ($n = 7$) were without effect (Fig. 8). Thus, duodenal field stimulation involves an orally projecting chain of cholinergic neurones with nicotinic synapses.

In three experiments, stimulation via additional electrodes placed 8–9 cm distal to the pylorus still caused pronounced excitation at the pylorus, whereas no clear activation occurred from electrodes placed at the ligament of Treitz (20–28 cm distal to the pylorus).

Pyloric motor responses to antral field stimulation and antral activity

Antral field stimulation inhibited pyloric phasic and tonic activity, abolished spontaneous pyloric activity (Fig. 9*A*) and pyloric activation due to duodenal field stimulation (Fig. 9*B*). This inhibitory response occurred without affecting antral motility, at a stimulation frequency of 0·5 Hz, but field stimulation of the antrum at frequencies higher than 1 Hz caused strong antral contractions after a short latency period. These contractions were detected by the antral strain gauges and manometrically by the antral side hole and the sleeve sensor. Consequently, the experiment did not determine whether pyloric inhibition was still induced in the presence of antral activation at frequencies above 1 Hz.

The data on the inhibitory effect of antral field stimulation on the excitatory effect of duodenal field stimulation are presented in Table 2. This inhibitory effect due to antral field stimulation (1·0 Hz) was not blocked by atropine ($n = 7$), hexamethonium ($n = 5$) or both ($n = 3$) nor by propranolol ($n = 6$), but was abolished by intra-arterial application of 50–100 μg TTX to the pyloric region. A quantitative analysis for atropine and hexamethonium was not possible as both blocked the pyloric response to duodenal field stimulation. After phentolamine ($n = 7$) the inhibitory effect of antral field stimulation was reduced but not completely abolished.

When spontaneous antral contractions occurred they also inhibited the pyloric motility (Fig. 3*C*). Similar antral contractions stimulated by close intra-arterial injection of acetylcholine (0·1–1 μg I.A.) to the antrum were also associated with inhibition of pyloric motor activity (results not shown).

Influence of antral and duodenal transection on pyloric motor responses to vagal stimulation and antral and duodenal field stimulation

The pyloric inhibitory effect of antral field stimulation (1–3 Hz, 0·5 ms) was abolished by antral transection between the stimulating electrodes and the pylorus ($n = 3$). The transection level was approximately 4 cm orad to the pylorus. A second pair of electrodes implanted in the antrum distal to the transection line continued to produce pyloric inhibition after antral transection (Fig. 10*A*). The antral transection did not block either antral contractions in response to vagal stimulation or the inhibitory effect of vagal stimulation (3 Hz, 3 ms) on pyloric motility (Fig. 10*A*).

The excitatory effects of duodenal field stimulation (0·2–3 Hz, 0·5 ms) were abolished by duodenal transection between the stimulus electrodes and the pylorus. The transection level was approximately 3 cm aborad to the pylorus. A second pair of electrodes implanted proximal to the duodenal transection line continued to produce pyloric excitation after transection (Fig. 10*B*). The vagal inhibitory and excitatory effects on the pylorus were not affected by duodenal transection alone or in combination with antral transection.

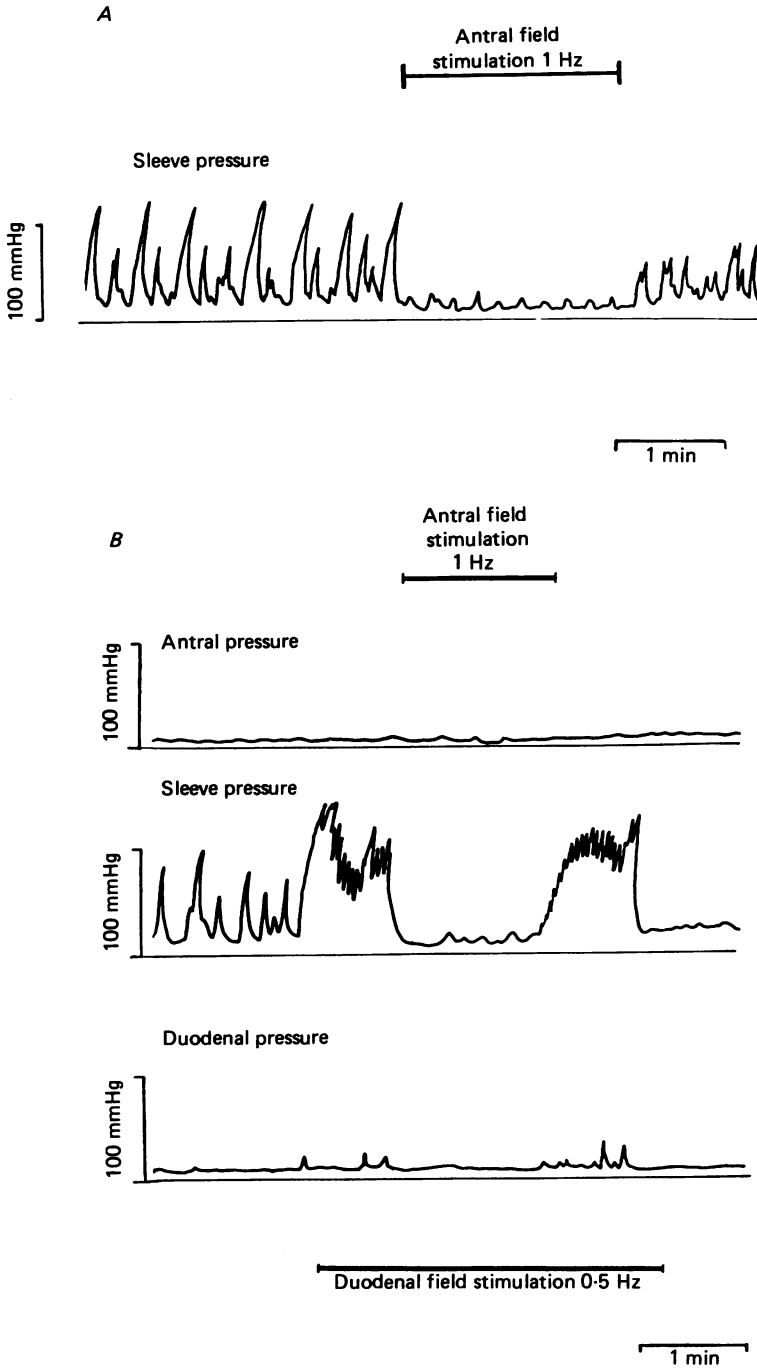


Fig. 9. Dynograph recording of the effect of antral field stimulation (1 Hz, 0·5 ms, 40 V) on spontaneous pyloric motor activity (*A*) and pyloric motor activity due to duodenal field stimulation (*B*). There were submaximal contractions of the antrum recorded at the strain gauges (not shown) in response to antral field stimulation.

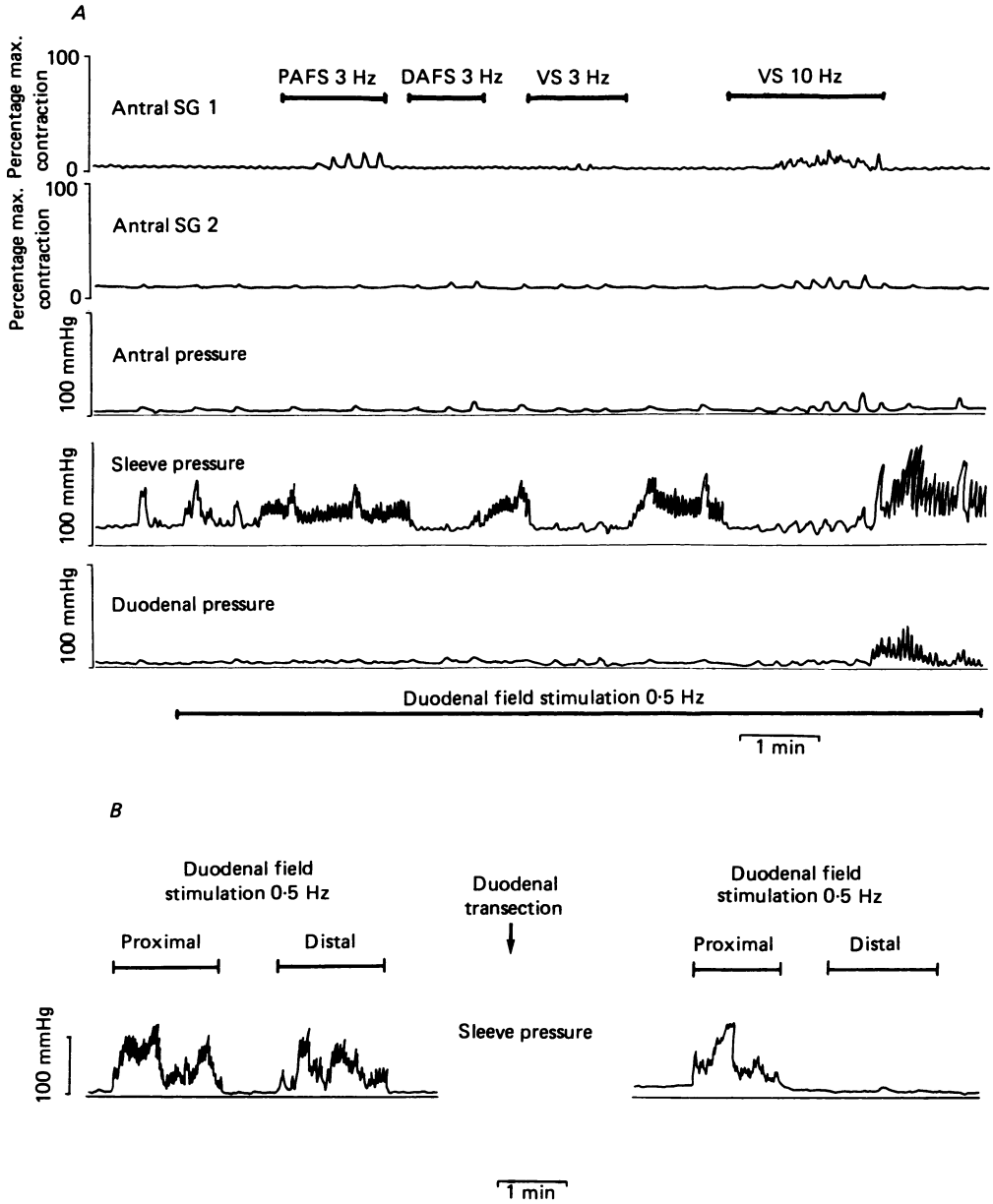


Fig. 10. *A*, dynograph record showing the effect of antral field stimulation and vagal stimulation on stimulated pyloric motor activity after antral transection between the proximal (Antral SG 1) and distal (Antral SG 2) antral strain gauges. The control electrodes aborad to the transection and vagal stimulation still elicit inhibitory responses. Note the excitatory response to vagal stimulation on both sides of the transection. *B*, effect of duodenal field stimulation (0·5 Hz) on pyloric motor activity before and after transection between the proximal and distal duodenal stimulation electrode. The effects of the distal electrode are blocked after transection. Proximal antral field stimulation, PAFS; distal antral field stimulation, DAFS; vagal stimulation, VS.

DISCUSSION

This study has demonstrated the existence of a basal pressure pattern in the pylorus, even after neural blockade with TTX, indicative of myogenic activity. Very low frequencies of vagal stimulation elicit pyloric excitation via a nerve pathway involving muscarinic and nicotinic synapses. In addition it has been shown for the first time that duodenal field stimulation is a potent stimulus for pyloric contractions, which is mediated by a chain of orally projecting cholinergic nerves with nicotinic and muscarinic synapses. Finally it has been demonstrated that antral field stimulation causes a potent inhibition of pyloric motility, mediated in part by non-cholinergic, non-adrenergic mechanisms and in part by a phentolamine-sensitive mechanism presumably involving α-adrenergic receptors. This is the first indication of the possible existence of an inhibitory intramural neural pathway from the antrum to the pylorus.

Myogenic control of the pylorus

A basal tone, evident as a zone of increased pressure between the antrum and duodenum, has been shown to exist in the pylorus of fasted chloralose–urethane-anaesthetized dogs and may regulate flow from the antrum to the duodenum and reflux from the duodenum to the antrum. The results, however, must be interpreted in the knowledge that the regulatory mechanisms may be altered by the experimental conditions, especially anaesthesia, and by local manipulation. Nevertheless, in a study using a balloon-withdrawal method, Brink *et al.* (1965) demonstrated a narrow zone of raised pressure at the gastroduodenal junction in fasted conscious dogs.

The pyloric region showed a characteristic pattern of basal activity. Individual contractions in the basal state often showed a biphasic pattern very similar to the electrical activity recorded *in vivo*, using extracellular suction or intracellular electrodes, from the gastroduodenal junction in the rhesus monkey, cat, dog and baboon (Daniel, 1965; Bortoff & Davis, 1968). The similar pattern and frequency of slow waves and pyloric pressure waves supports the view that this form of pyloric contraction is primarily myogenic. This theory is further supported by the observation that basal pyloric tonic and phasic contractions persisted after application of TTX. TTX even increased the spontaneous activity suggesting a tonically active inhibitory neural innervation in the gastroduodenal junction. Atropine reduced the frequency and amplitude of the phasic pyloric waves in the basal state, suggesting that a tonic cholinergic influence may contribute to the activation of pyloric phasic contractions.

Three major questions arise from the conclusion that the isolated pyloric contractions, which occur in the basal state, are predominantly myogenic in origin: (1) which muscle structure is responsible for the isolated pyloric contractions? (2) how do the results correlate with previous studies on the electro-physiological characteristics and anatomical structures of the pylorus? and (3) what is the mechanism that leads to vigorous phasic contractions of the pyloric muscle when there is little or no contractile activity in the antrum or duodenum?

Studies of the anatomy of the pylorus indicate the presence of two muscle rings (Schardt & van der Zypen, 1974; Schulze-Delrieu & Shirazi, 1983; Cai & Gabella,

1984). The distal ring is about 0·5 cm wide and is the narrowest part of the pyloric canal. Recent manometric studies in humans, in which sleeve sensor measurements were combined with recordings from multiple side holes spaced at 3 mm intervals across the pylorus, suggested that the region producing the isolated phasic and tonic pyloric contractions is about 3–6 mm wide (Heddle *et al.* 1986). The present study, together with others (Brink *et al.* 1965; Behar *et al.* 1979; Heddle *et al.* 1986), suggests that the distal circular muscle ring is the anatomical structure responsible for the IPCs recorded in the present study. Studies using electron microscopy in the guinea-pig (Cai & Gabella, 1984) and light microscopy in the mouse (Schardt & van der Zypen, 1974) and dog (E. E. Daniel, M. Costa & J. Furness, unpublished) have shown that this circular muscle ring has a very rich neural innervation and a distribution of neuropeptides which differs from other components of the pyloric and antral musculature. The pyloric inner muscle ring of the dog shows a very high density of immunoreactivity for enkephalins, vasoactive intestinal polypeptide (VIP), substance P and neuropeptide Y (NPY) compared to the antrum and duodenum (E. E. Daniel, M. Costa & J. Furness, unpublished). Furthermore, recent ultrastructural studies in the guinea-pig (Cai & Gabella, 1984) have shown that the circular muscle of the pylorus is separated from that of the duodenum by a connective tissue septum of about 100–200 μm in width. In contrast to this structural separation of the inner ring of pyloric muscle, there is continuity of the myenteric plexus and most of the gastric longitudinal muscle layer between the stomach and duodenum.

The electrophysiology of the gastroduodenal region has been studied extensively using intracellular (Daniel, 1965) and extracellular (Bass, Code & Lambert, 1961; Bortoff & Weg, 1965; Bortoff & Davis, 1968) electrode recording techniques. Bass *et al.* (1961) found that electrical activity from the pyloric canal or duodenal bulb 'was greatly attenuated or lost in the pylorus'. However, studies on cat tissue *in vitro* (Bortoff & Davis, 1968) and in the dog and other species *in vivo* (Bortoff & Weg, 1965; Daniel, 1965; Bortoff & Davis, 1968) showed that slow waves could be transmitted across the pylorus in both directions. Therefore, there is electrophysiological data which supports the suggestion that basal pyloric contractions of myogenic origin are driven partly by antral and partly by duodenal slow waves.

As a working hypothesis, it is suggested that slow waves from the antrum and duodenum could, under appropriate conditions, be additive and thus generate action potentials and contractions during the variable pattern of phasic pyloric contractions observed. Alternatively, a prolonged depolarization of this muscle or a prolonged second component of the action potential has been reported *in vitro* from this muscle (Szurszewski, 1981). During the prolonged depolarization excitatory junction potentials or electrotonic propagation of depolarizing events may be capable of initiating spikes. Thus multiple contractions could be superimposed on a single slow wave or action potential.

Neural control of pylorus: vagus nerve

The results indicate that neural influences can alter pyloric motility through several mechanisms. Under the conditions of the present experiments, cervical vagal transection showed no consistent effect on pyloric motility, a result consistent with

the studies of Langley (1898) in anaesthetized cats. Vagal stimulation, at different frequencies, showed that the vagi contain nerve fibres which can excite and inhibit the pylorus. Whereas pyloric inhibition is evident at stimulation frequencies > 0·7 Hz, frequencies of 0·6 Hz or less produce phasic pyloric contractions synchronous with each stimulus, superimposed on an overall inhibition of basal pyloric motility. The nerve pathway mediating the excitatory response to vagal stimulation involves muscarinic and nicotinic components.

Increasing stimulus frequencies gradually diminish the excitatory effect of vagal stimulation and produce an inhibition of pyloric function. Over a narrow range of frequencies and pulse widths, this inhibition was obtained without activation of the adjacent antral or duodenal muscles. Electrical stimulation of the distal vagal stumps at a frequency > 1 Hz caused pyloric inhibition which was followed within a few seconds by antral contractions. This inhibitory effect proved to be non-adrenergic and non-cholinergic in nature, since atropine, phentolamine and propranolol failed to block the response, a result consistent with those of Langley (1898) and Mir *et al.* (1979) *in vivo* and *in vitro* (Anuras *et al.* 1974). Studies of electrical activity of the antrum and pylorus in the rabbit suggested that there is a pathway in the dorsal vagal trunk which controls a non-adrenergic, non-cholinergic inhibitory innervation to the pylorus (Deloof & Rouseau, 1985) which might be involved in the relaxation of this region. Endogenous opioid peptides are unlikely to be involved in the pathway as naloxone did not block the inhibitory response to either antral or vagal stimulation (Allescher, Ahmad, Daniel, Dent, Kostolanska & Fox, 1987).

The existence of two response patterns, to different parameters of vagal stimulation, suggests the existence of two types of vagal fibres with opposing effects on gastric motility, as was suggested by Martinson & Muren (1963) in studies in the cat. They found evidence for 'low-threshold' excitatory nerve fibres which were blocked by atropine and 'high-threshold' fibres which produced gastric inhibition via a non-cholinergic mechanism and concluded that the inhibitory fibres had a considerably smaller diameter than the excitatory fibres (Martinson & Muren, 1963).

The apparent existence of an excitatory vagal influence on the pylorus has been reported in a previous study in which pyloric motility was measured by serosal strain gauges sutured to the antral and pyloric region (Mir *et al.* 1979). However, in this study, very high stimulus frequencies (16 Hz, 5 ms) were needed to obtain the excitatory response, while in the present experiments stimulation at these frequencies led to vigorous antral contractions which made it impossible to record selectively from the pyloric region. Whilst it can be suggested that higher frequencies of vagal stimulation also inhibit pyloric activity, the possibility cannot be excluded that phasic contractions, stimulated by high-frequency vagal stimulation, lead to pyloric excitation.

Neural control of pylorus: duodenal intrinsic nerves

Electrical field stimulation of the duodenal intramural nerves 3–5 cm aborad to the pylorus caused an almost immediate pyloric contraction which was especially pronounced when the level of basal pyloric motility was low. This response was not

influenced by prior vagotomy. At stimulus frequencies of 0·7 Hz and less, the pylorus also contracted with each pulse. The results from pharmacological interventions (response blocked by atropine or hexamethonium) and duodenal transection indicate that this excitatory pyloric response is due to a chain of cholinergic neurones projecting in the orad direction. This, in common with a similar effect due to vagal stimulation, is a surprising finding since the pathway appears multineuronal but not to require temporal summation for transmission. Interestingly, this direct stimulation–contraction relationship was observed in some experiments up to 0·7–1·0 Hz and consequently caused up to forty-two to sixty phasic contractions of the pylorus per minute. This effect is believed to be a neural phenomenon for the following reasons. First, the stimulation site was 3–5 cm apart from the pylorus. Second, earlier studies by Sarna & Daniel (1973, 1976) showed that direct electrical stimulation could only drive antral contractions up to a rate of 10 min^{-1} and duodenal contractions up to 26 min^{-1} and the intervening duodenum did not show this high frequency of contractions. Third, the response was blocked by atropine, hexamethonium, TTX and duodenal transection orad to the stimulus electrodes. The pyloric muscle, thus, seems to have the ability to contract to neural stimulation with a refractory period of less than 2 s; i.e. at a rate faster than slow waves in the region. This implies that the pyloric muscle, unlike antrum can be driven to a frequency greater than 9–10 min^{-1} (Sarna & Daniel, 1973), similarly its driven frequency can exceed that of the duodenum (25–26 min^{-1}) (Sarna & Daniel, 1976). Stimulation at higher frequencies caused an irregular pattern of strong pyloric phasic contractions occasionally interposed with periods of no phasic contractile activity. As a result, the pyloric contractile response to duodenal field stimulation diminished at frequencies greater than 0·5 Hz, suggesting that duodenal field stimulation at higher frequencies could also activate an inhibitory pathway, from the duodenum to the pylorus, which causes the irregular pattern of phasic activity and decreases tonic activity.

Several previous studies have suggested a regulation of pyloric motor activity originating from the duodenum or the jejunum (Fisher & Cohen, 1973; Cooke, 1977; Heddle *et al.* 1986). Stimuli such as intraluminal amino acids (Fisher & Cohen, 1973; Cooke, 1977), hypertonic solutions (Cooke, 1977), hydrochloric acid (Fisher & Cohen, 1973; Valenzuela, Defilippi & Csendes, 1976; Cooke, 1977; Reynolds *et al.* 1984) and lipids (Fisher & Cohen, 1973; Heddle *et al.* 1986) have been shown to stimulate pyloric activity. In the study by Valenzuela *et al.* (1976), pyloric activation induced by intraduodenal infusion of hydrochloric acid was completely abolished by atropine, indicating the presence of a pathway which may correspond to that which was activated by duodenal field stimulation in the present study.

Neural control of pylorus: antral intrinsic nerves

In contrast to the effects of duodenal field stimulation, antral field stimulation elicited a very potent inhibitory effect on pyloric motor function, abolishing phasic isolated pyloric contraction and reducing tonic activity when present. This inhibitory antral pathway involves intramural nerves, since muscular transection of the antrum abolished the inhibitory response, while atropine and hexamethonium were without effect. A quantitative evaluation of the effect of atropine and hexamethonium on the inhibition of duodenal field-stimulated pyloric responses by antral field stimulation

was not possible, since both atropine and hexamethonium blocked the excitatory effect of duodenal field stimulation. Spontaneous or pharmacologically induced antral contractions also caused inhibition of pyloric motility. This inhibitory pathway may contribute, together with vagal inhibitory fibres, to the tonic inhibitory innervation of the pylorus which has been inferred from the finding that inhibition of neural responses with TTX led to increased basal pyloric motility (Telford, Mir, Mason & Ormsbee, 1979; Lerman *et al.* 1981; the present study).

Relation to other studies

The intramural antral and duodenal pathways demonstrated in these experiments could be responsible for the modulation of pyloric motility previously described (Weisbrodt, Wiley, Overholt & Bass, 1969; Fisher & Cohen, 1973; Cooke, 1977). The potency and the consistency of both the excitation from the duodenum and the inhibition from the antrum, demonstrated in this study, suggests that these effects are of physiological importance in the regulation of transpyloric flow in both directions. Possible impairment in the function of these pathways could contribute to both abnormal gastric emptying (Mearin, Camilleri & Malagelanda, 1986) and abnormally frequent duodenogastric reflux.

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